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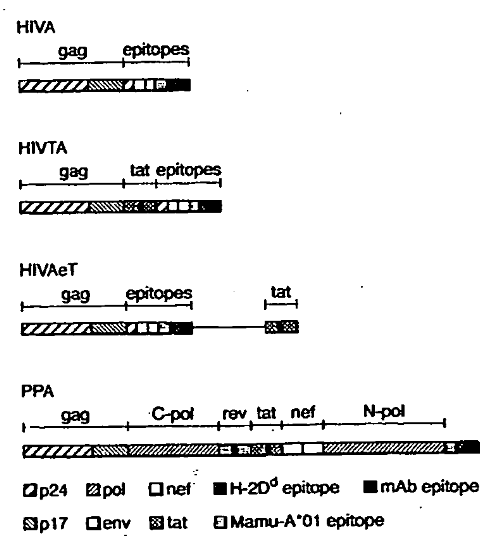
(54) Title: IMPROVEMENTS IN OR RELATING TO IMMUNE RESPONSES TO HIV



(57) Abstract: Disclosed is an immunogen in sterile form suitable for administration to a human subject, the immunogen comprising: at least a portion of the gag protein of HIV, said gag protein being from an HIV clade or having a consensus sequence for one or more HIV clades, and comprising at least parts of p17 and p24; and a synthetic polypeptide comprising a plurality of amino acid sequences, each sequence comprising a human CTL epitope of an HIV protein, and wherein a plurality of HIV proteins are represented in the synthetic polypeptide, said CTL epitopes being selected to stimulate an immune response to one or more HIV clades of interest.

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Title: **Improvements in or Relating to Immune Responses to HIV**

Field of the Invention

This invention relates to an immunogen designed to elicit an anti-HIV immune response in a human subject (especially a cell-mediated response), a nucleic acid molecule encoding the immunogen, compositions comprising the immunogen and/or the nucleic acid molecule, and to a method of inducing an anti-HIV immune response (especially a cell-mediated response) in a human subject.

Background of the Invention

Development of effective human immunodeficiency virus (HIV) vaccines is one of the primary goals of current acquired immunodeficiency syndrome (AIDS) research. Despite progress in prevention and powerful drug combinations to treat HIV infection, an estimated 16,000 people become infected every day. Over 90% of new infections occur in developing countries for which the recent medical advances are not immediately applicable or affordable. The best hope for these countries is the development of an effective, accessible HIV vaccine. There is now growing optimism among scientists that an AIDS vaccine may be possible (McMichael & Hanke 1999 Nat. Med. 5, 612-614; Gold 1999 IAVI Report 4, pp 1-2, 8-9, 15-16 & 18).

An ideal prophylactic vaccine should induce sterilizing immunity, so that after exposure, the virus would never be detected in the body. However, this is probably an unrealistic objective. Rather, an attainable goal may be a vaccine-induced immunity that results in a limited and transient virus replication, after which the virus becomes undetectable, there are no signs of disease and no transmission to other individuals. Alternatively, a potentially successful vaccine may induce immune responses that at least hold the virus in check at levels so low, that both progression to AIDS and transmission are entirely or substantially prevented.

To induce sterilizing immunity, a prophylactic vaccine may need to elicit both humoral and cell-mediated immune responses. Since HIV was isolated and sequenced, there has been a considerable effort to develop envelope-based vaccines inducing neutralizing antibodies (nAb). However, this has proved to be exceedingly difficult (Heilman & Baltimore 1998 Nat. Med. 4 (4 Suppl.) 532-534). Although some success was reported in inducing nAb against laboratory HIV strains (Berman *et al*, 1990 Nature 345, 622-625; Fultz *et al*, 1992 Science 256, 1687-1690), it has been extremely difficult to neutralize primary isolates (Trkola *et al*, 1998 J. Virol. 72, 1876-1885; Haynes 1996 Lancet 34, 933-937). An explanation for the first 15 years of relative failure has been provided by the crystal structure of the core gp 120, which revealed multiple mechanisms by which HIV prevents efficient induction of nAb (Wyatt *et al*, 1998 Nature 393, 705-711; Kwong *et al*, 1998 Nature 393, 638-659). As a result of these difficulties, the emphasis of many vaccine designers has shifted to the induction of cell-mediated immune responses, which are mediated (predominantly) by cytotoxic T lymphocytes.

Cytotoxic T lymphocytes (CTL) are usually $CD8^+$ cells and participate in an organism's defence in at least two different ways: they kill virus-infected cells; and they secrete a variety of cytokines and chemokines that directly or indirectly contribute to the suppression of virus replication. CTL-mediated protection after vaccination may depend on the levels of CTL present in the circulation and, perhaps, specifically for proteins expressed early (regulatory proteins) rather than late (structural proteins) in the replication cycle.

The induction and maintenance of $CD8^+$ T cell responses require "help" provided by $CD4^+$ T lymphocytes (helper T cells). In some HIV-infected individuals, high levels of HIV-specific helper response have been detected.

Identification of methods for induction of strong $CD8^+$ T cell responses would provide tools for studying their role(s) in shaping the course of HIV infection and may stimulate progress towards an effective HIV vaccine. Previously, a prototype HIV vaccine was constructed as a string of partially overlapping epitopes recognised by murine, macaque and human CTL, which was delivered by vaccine vehicles that were safe and acceptable for use in humans, a DNA vector and modified vaccinia virus Ankara (MVA) vector (Hanke *et al*, 1998 Vaccine

16, 426-435; Hanke *et al*, 1998 J. Gen. Virol. 79, 83-90). In mice, the most potent protocol for induction of CTL was found to be DNA priming followed by MVA boosting (Hanke *et al*, 1998 Vaccine 16, 439-445; Schneider *et al*, 1998 Nat. Med. 4, 397-402) that is, priming mice with nucleic acid encoding the relevant polypeptide, followed by boosting the mice by inoculation with a modified vaccinia virus Ankara ("MVA") vector expressing the relevant epitopes.

WO 98/56919 discloses a "prime-boost" vaccination strategy, involving (i) priming with a composition comprising a source of one or more T cell epitopes of a target antigen, together with a pharmaceutically acceptable carrier, and (ii) boosting with a composition comprising a source of one or more T cell epitopes of the target antigen, including at least one T cell epitope that is the same as a T cell epitope of the priming composition.

The present invention aims, *inter alia*, to provide immunogens which may be useful in eliciting an HIV-specific response in humans. All documents and publications mentioned in this specification are incorporated herein by reference.

Summary of the Invention

In a first aspect the invention provides an immunogen in sterile form suitable for administration to a human subject, the immunogen comprising: at least a portion of the gag protein of HIV, said gag protein being from an HIV clade or having a consensus sequence for one or more HIV clades, and comprising at least parts of p17 and p24; and a synthetic polypeptide comprising a plurality of amino acid sequences, each sequence comprising a human CTL epitope of an HIV protein, and wherein a plurality of HIV proteins are represented in the synthetic polypeptide, said CTL epitopes being selected to stimulate an immune response to one or more HIV clades of interest.

For present purposes "sterile" refers to the general absence of viruses, bacteria, fungi, yeasts, chlamydia, mycoplasma, and spores of any of the foregoing (especially microbes pathogenic in humans). However, the immunogen may comprise one or more specific known microbial (e.g. viral or bacterial) vectors which serve to express the gag protein and/or synthetic polypeptide in a human subject. Such vectors are well known to those skilled in the art and

include, for example, viruses such as adenoviruses and pox viruses which are inherently non-pathogenic in humans or have been subjected to genetic manipulation or other modification to render them non-pathogenic in humans. Particularly preferred is vaccinia virus, especially modified vaccinia virus Ankara (MVA). Other specific preferred viral vectors include Semliki Forest Virus (SFV) and Sindbis virus (see Smerdon & Liljeström 2000, Gene Ther. Regul. 1, 1-31). Suitable bacterial vectors include BCG, and attenuated strains of Salmonella Spp. (especially "double aro" mutants of Salmonella which are being developed as vaccines for diarrhoeal diseases), and Shigella (see Shata *et al*, 2000 Mol. Med. Today 6, 66-71).

Other expression systems, which may be useful for producing the immunogen include a tobacco mosaic virus (TMV) expression vector (Palmer *et al*, 1999 Arch. Virol. 144, 1345-60) and NS1 tubules of bluetongue virus (Adler *et al*, 1998 Med. Microbiol. Immunol. (Berl.) 187, 91-96).

The term "synthetic" as used herein, is intended to refer to a polypeptide which is not, in its entirety, present in any naturally-occurring HIV isolate. The term "synthetic" is not intended to indicate that the polypeptide is necessarily synthesised by conventional chemical techniques of solid phase peptide synthesis. Whilst this represents one possibility, it is generally to be preferred that the polypeptide is synthesised by transcription and/or translation from an appropriate nucleic acid molecule encoding the synthetic polypeptide. Such methods of synthesis are well known to those skilled in the art and form no part of the invention.

It is preferred that the gag protein (or portion thereof) and the synthetic polypeptide are joined in some way on a single entity. For example, a viral vector may express both the gag protein and the synthetic polypeptide as separate components of the immunogen. Alternatively, both components of the immunogen may be covalently coupled or conjugated to each other or to a common carrier entity (such as a liposome, ISCOM or molecule). In preferred embodiments, both the gag protein and the synthetic polypeptide components of the immunogen are present in a single polypeptide or fusion protein. In such a fusion protein, the gag protein and synthetic polypeptide may be essentially the only components present. Alternatively the fusion protein may comprise other components which may

correspond to other HIV antigens (or portions thereof), or may be derived from other sources. Thus, in some embodiments, the polypeptide will comprise a portion of the gag protein substantially adjacent to the synthetic polypeptide (i.e. with fewer than 10 intervening amino acid residues). In other embodiments, the portion of the gag protein and the synthetic polypeptide may be separated by one or more intervening components (i.e. with 10 or more intervening amino acid residues), which will typically comprise one or more further HIV antigens.

It is also generally preferred that the immunogen does not contain the entire gag protein amino acid sequence. Typically between 55 and 95% of the gag protein will be present, preferably between 65 and 85%, most preferably about 75%.

The wild type HIV gag protein is known to consist of three portions termed p17, p24 and p15. These are synthesised in infected cells as a single polyprotein, with p17 at the N terminus. Normally, in HIV-infected cells, the N terminus of p17 is myristylated.

It is desirable that the gag portion of the immunogen will comprise at least part of p17 and p24, but it is generally preferred that the p17 component will be modified in some way to prevent myristylation. Conveniently this can be accomplished by reversing the order of the p17 and p24 components in the immunogen, such that p17 is no longer at a free N terminus and cannot therefore be myrisylated. The inventors believe that this may improve the efficiency of presentation of peptides, derived from the immunogen, to a subject's immune system.

The gag protein component of the immunogen will generally comprise at least one T-helper cell, HLA Class II-restricted, peptide epitope and preferably comprise a plurality of such epitopes (preferably such that a number of different HLA Class II-restricted alleles are represented in the gag component). The gag protein component will also typically comprise one or more CTL HLA Class I-restricted peptide epitopes.

The synthetic polypeptide component of the immunogen will conveniently take the form of a string of CTL epitopes, each represented by, or contained within, a respective sequence of about 8-12 amino acids. Desirably at least some (preferably most) of the epitopes will be partially overlapping (such that one or more amino acids of one epitope will also be

contained within the sequence of an adjoining epitope). Some "non-epitopic" amino acid sequence may be present between neighbouring epitopes, but this is generally to be avoided.

Non-epitopic amino acid sequence between neighbouring epitopes is preferably less than 20 amino acid residues, more preferably less than 10 residues, and most preferably 1-5 amino acid residues. It will be apparent that such non-epitopic amino acid sequence may comprise linkers, spacers and the like which optimise the expression levels of the synthetic polypeptide or optimise its immunogenicity.

Thus in one extreme embodiment, all of the human CTL epitopes in the synthetic polypeptide may be overlapping and, at the other extreme, every epitope may be separated from its neighbours by at least some non-epitopic amino acid sequence. It is generally to be preferred that at least 50% of the human CTL epitopes are overlapping.

In addition to overlapping epitopes, the synthetic polypeptide may comprise at least some "adjacent epitopes". The term adjacent epitopes refers to epitopes which are not overlapping but which are not separated by any intervening non-epitopic amino acid sequence.

Thus in preferred embodiments the synthetic polypeptide comprises a mosaic pieced together from small (typically about 10-20 amino acid residue) fragments of different HIV proteins, which fragments will typically comprise one, two or three known adjacent and/or overlapping human CTL epitopes. Where a plurality of fragments are present in the synthetic polypeptide from the same HIV protein, the fragments will typically have been selected from discontinuous portions of the protein, so that it is unlikely that the synthetic polypeptide comprises a sequence corresponding to more than 20-25 consecutive amino acid residues of a particular HIV protein. Generally the synthetic polypeptide is designed so as essentially to omit those portions of HIV proteins not known to contain any human CTL epitopes.

A plurality of different HIV proteins will preferably be represented in the synthetic polypeptide. The synthetic polypeptide may contain epitopes present in any HIV antigen, but preferably will comprise at least one epitope present in one or more of the following: p24; pol; gp41; gp120; and nef. In one preferred embodiment, the synthetic polypeptide

comprises at least one CTL epitope present in each of the aforementioned HIV proteins. The synthetic polypeptide may additionally comprise at least one CTL epitope present in each of the following HIV proteins: vpr, vpu, vif (and especially) tat and rev.

It will be understood that the term "human CTL epitope" as used herein refers not to the origin of the protein from which the epitope derives, but indicates that the epitope is recognized and responded to by the CTL of at least a portion of the human population. Typically a human CTL epitope will be recognized by at least 0.01%, preferably 0.1%, and more preferably at least 1% of the world's human population.

In one particular embodiment, the immunogen specifically excludes any epitope from the env protein generally recognised by the human immune system. Most presently-used diagnostic tests are based on detection of an HIV env-specific immune response, so by excluding env components from the immunogen it is possible to distinguish between immune responses rising from infection with the virus and inoculation with the immunogen.

In one embodiment, the CTL epitopes present in the synthetic polypeptide are selected such that an immune response to HIV clade A will be generated. Preferably however, the synthetic polypeptide is large enough, and the CTL epitopes appropriately selected, such that an immune response which is cross-reactive against different HIV clades will be stimulated. This can conveniently be achieved by including one or more CTL epitopes which are conserved among different HIV clades. Several such epitopes are known to those skilled in the art (see Table 1 below).

In a preferred embodiment, the immunogen comprises at least one epitope (conveniently a CTL epitope) which is recognized by one or more laboratory test mammals, (e.g. mouse and/or monkey). Such an epitope can readily be incorporated within the synthetic polypeptide. Inclusion of an epitope of this sort allows for the quality, reproducibility and/or stability of different batches of the immogen to be assayed in a potency assay using the laboratory test mammal (such as a mouse or macaque monkey). Examples of such epitopes include the amino acid sequence ACTPYDINQML (Seq. ID No. 1; containing a dominant epitope derived from simian immunodeficiency virus, SIV, gag p27, recognised by rhesus

macaque monkey CTLs) and RGPGRAFVTI (Seq. ID No. 2; an H-$2D^d$-restricted CTL mouse epitope derived from HIV env protein).

A list of some 23 human CTL epitopes suitable for inclusion in the synthetic polypeptide is shown in Table 1. The list is not exhaustive. A preferred immunogen will comprise at least 10 of such human CTL epitopes, more preferably at least 15, most preferably at least 20. In a particular embodiment each of the 23 human CTL epitopes listed in Table 1 will be represented in the synthetic polypeptide, optionally together with the macaque and murine epitopes also listed in Table 1.

Desirably the immunogen may additionally comprise a small tag or marker. Such a tag or marker should be as small as possible, in order to minimise the amount of extraneous material present in the immunogen. A convenient tag or marker allows for detection of expression and/or quantification of the amount of immunogen. Suitable tags include epitopes recognized by the monoclonal antibody Pk (Hanke *et al*, 1992 J. Gen. Virol. 73, 653-660).

The immunogen of the invention will conveniently be mixed with other substances in order to provide a vaccine composition. For example, a vaccine composition may additionally comprise one or more of the following: an adjuvant (e.g. alum), liposome, or immunostimulatory complex (ISCOM). A vaccine will also generally comprise a sterile diluent, excipient or carrier, such as a physiologically acceptable liquid (e.g. saline or phosphate-buffered saline solutions). The vaccine may be presented as a liquid or, more preferably, as a freeze-dried solid, which is suspended or dissolved in a physiologically acceptable liquid prior to administration.

Methods of administering the immunogen to a human subject will be apparent to those skilled in the art. Such methods include, in particular, intramuscular injection, subcutaneous injection, or delivery through the skin by needleless injection device. Alternatively, vaccines (especially those comprising bacterial vectors e.g. attenuated Salmonella or Shigella spp.) may be given orally, intranasally or by any other suitable route. A suitable dose of immunogen may typically be from 1 to 500 mg, typically from 10 to 100mg, depending on the size of the immunogen, the body mass of the recipient etc. A suitable dose can, if necessary, be ascertained by routine trial-and-error with different groups of subjects being

given different doses of immunogen, and the immune response of the subjects to the immunogen asayed in order to determine optimum dose size. The immune response of the subjects can be assayed by conventional immunological techniques (e.g. chromium release assay, using peripheral blood lymphocytes obtained from subjects' blood samples, acting on peptide-pulsed or virus-infected chromium-labelled target cells).

In a second aspect, the invention provides a nucleic acid molecule encoding a fusion protein, the fusion protein comprising an immunogen in accordance with the first aspect of the invention defined above.

The nucleic acid molecule is preferably in isolated, sterile form, suitable for administration to a human subject. The nucleic acid molecule is preferably "humanized" i.e. employs codons to code particular amino acids, which codons are frequently used in highly expressed human genes (Andre *et al*, 1998 J. Virol. 72, 1497-1503), instead of those codons used by HIV.

Desirably the nucleic acid molecule is contained within a vector, the sequence encoding the immunogen being operably linked to a promoter sequence active in human cells. Conveniently the promoter is a strong viral promoter such as the promoter from human cytomegalovirus (CMV). The vector will preferably also comprise an enhancer and polyadenylation signals, which are functional in human cells. Ideally, for maximum safety, the vector should not contain any origin of replication functional in human cells, to prevent undesirable replication of the vector.

The vector may be administered to the subject in isolation, as essentially 'naked' nucleic acid (preferably DNA), or else may be packaged within a delivery means, such as a virus, bacterium, liposome, or gold-coated particles and the like. One suitable delivery means is the modified vaccinia virus Ankara (MVA): the immunogen gene or open reading frame (ORF) may be inserted into MVA, for example, at the thymidine kinase locus.

It will be appreciated by those skilled in the art that a vector may comprise a nucleic acid encoding an immunogen (the nucleic acid therefore being in accordance with the second aspect of the invention), and that the vector may also possess, at its surface or internally, the polypeptide immunogen in accordance with the first aspect of the invention.

Whether administered as naked nucleic acid, or packaged within a delivery means, at least some of the administered nucleic acid enters the cells of the subject and is transcribed (if necessary) and translated, resulting in the *in situ* synthesis of the immunogen in the subject, who then develops an immune response to the immunogen.

As with the immunogen preparation *per se*, a nucleic acid encoding the immunogen may be administered to a human subject by any of a number of known routes e.g. subcutaneous or intramuscular injection, oral delivery, or delivery through the skin by means of needleless injector. Particular examples of administration by needleless injection device are disclosed in WO 97/34652 and WO 97/48485. A suitable dose of nucleic acid may be from 10μg to 10mg, typically from 100μg to 1mg, depending on the size of nucleic acid molecule, route of administration, body mass of the recipient etc. Again, routine trial and error (with the benefit of the present teaching) will enable those skilled in the art to determine an optimum dose of the nucleic acid.

Successful delivery of DNA to animal tissue has been achieved by cationic liposomes [Watanabe et al., Mol. Reprod. Dev. 38:268-274 (1994); Sharkey *et al*, WO 96/20013], direct injection of naked DNA into animal muscle tissue [Robinson et al., Vacc. 11:957-960 (1993); Hoffman et al., Vacc. 12:1529-1533; (1994); Xiang et al., Virol. 199:132-140 (1994); Webster et al., Vacc. 12:1495-1498 (1994); Davis et al., Vacc. 12:1503-1509 (1994); and Davis et al., Hum. Molec. Gen. 2:1847-1851 (1993)], and embryos [Naito et al., Mol. Reprod. Dev. 39:153-161 (1994); and Burdon et al., Mol. Reprod. Dev. 33:436-442 (1992)], or intradermal injection of DNA using "gene gun" technology [Johnston et al., Meth. Cell Biol. 43:353-365 (1994)].

For DNA-based vaccination, delivery by injection of naked plasmid DNA has shown potential in mouse models for inducing both humoral and cellular immune responses. However, in larger animals, using DNA delivery for vaccination has been hampered by requiring large amounts of DNA or inducing persistent expression of an antigen with the potential for developing tolerance to the antigen. Berglund reported a strategy for inducing or enhancing an immune response by injecting mice with plasmid DNA

containing an alphavirus DNA expression vector having a recombinant Semliki Forest Virus (SFV) replicon in a eukaryotic expression cassette [Berglund et al., Nature Biotechnol. 16:562-565 (1998)]. The eukaryotic expression cassette controlled expression of the primary nuclear transcription of the SFV replicon. This SFV replicon transcript, encoding the heterologous antigen, was transported to the cytoplasm and amplified by the self-encoded SFV replicase complex. The amplified RNA replicon lead to high level production of an mRNA encoding the heterologous antigen. Similar results were described by Polo and his group [Polo et al., Nature Biotechnol. 16:517-518 (1998); Hariharan et al., J. Virol. 72:950-958 (1998)]. Both groups found strong immune responses could be induced using small amounts of input plasmid DNA.

Alternatively, a method to deliver DNA to animals that overcomes the disadvantages of conventional delivery methods is by administering attenuated, invasive bacteria containing a bacterial DNA vector having a eukaryotic expression cassette encoding the gene to be expressed. For example, U.S. Patent No. 5,877,159 to Powell et al., describes live bacteria that can invade animal cells without establishing a productive infection or causing disease to thereby introduce a eukaryotic expression cassette encoding an antigen capable of being expressed by the animal cells.

In a third aspect the invention provides a method of stimulating an anti-HIV immune response in a human subject, the method comprising preparing an immunogen in accordance with the first aspect of the invention, or a nucleic acid molecule in accordance with the second aspect of the invention; and administering said immunogen or nucleic acid molecule to the subject.

Conveniently, the method comprises the administering both the immunogen and the nucleic acid molecule. In particular, the method preferably comprises one or more administrations of the nucleic acid molecule ("priming") followed at a suitable interval (e.g. 1 week to 4 months) by one or more administrations of the immunogen ("boosting"). Boosting may be performed, for example, by administering a replication-competent (e.g. attenuated virus or bacterium) or non-replicating vector comprising the immunogen and/or a nucleic acid

molecule encoding the immunogen. Preferably boosting is achieved by administering the immunogen as part of an MVA viral particle, which particle may advantageously comprise a nucleic acid encoding the immunogen.

In a preferred embodiments, performance of the method will result in the generation in the subject of a protective immune response such that, should the subject subsequently be exposed to HIV infection, the subject will not go on to develop the symptoms of AIDS associated with HIV infection.

In a fourth aspect the invention provides for use of an immunogen in accordance with the first aspect of the invention and/or a nucleic acid in accordance with the second aspect of the invention in the preparation of a medicament to prevent or treat HIV infection in a human subject.

In a fifth aspect, the invention provides a nucleic acid sequence encoding the amino acid sequence shown in Figure 8A. Conveniently the nucleic acid comprises or essentially consists of the nucleotide sequence shown in Figure 8B.

In a sixth aspect, the invention provides a polypeptide comprising the amino acid sequence shown in Figure 8A.

The nucleic acid of the fifth aspect and/or the polypeptide of the sixth aspect may be used in immunogen/vaccine compositions as described in relation to the other aspects of the invention, and such immunogens and vaccines are accordingly considered within the scope of the invention, and may comprise vectors etc (especially MVA) as aforesaid. The invention further provides, in a seventh aspect, a method of stimulating an anti-HIV immune response in a human subject, the method comprising administering to the subject a nucleic acid in accordance with the fifth aspect of the invention and/or a polypeptide in accordance with the sixth aspect of the invention. Finally, the invention provides for use of a nucleic acid in accordance with the fifth aspect of the invention and/or a polypeptide in accordance with the sixth aspect of the invention in the preparation of a medicament to prevent or treat HIV infection in a human subject.

The invention will now be further described by way of illustrative example and with reference to the accompanying drawings, wherein:

Figure 1A is a schematic representation of immunogens, HIV A, HIVTA and HIVAeT in accordance with the invention; and an immunogen PPA;

Figure 1B shows the amino acid sequence of the HIVA immunogen (Seq. ID No. 26);

Figure 2A shows the nucleotide sequence (Seq. ID No. 27) of a nucleic acid (termed "HIVA gene") encoding the HIV A immunogen;

Figure 2B is a schematic representation of the method used to construct the HIVA gene;

Figure 3 is a schematic representation of a DNA vector molecule (pTHr. HIVA) in accordance with the invention, and the method of its construction;

Figure 4 is a micrograph showing immunofluorescent detection of HIVA expression by mouse cells following transfection with pTHr. HIVA;

Figures 5A, B & C are graphs showing the results of chromium release assays using a splenocytes obtained from mice inoculated with a DNA molecule or an immunogen in accordance with the invention;

Figure 6A shows the amino acid sequence of the HIV TA immunogen (Seq. ID No. 28);

Figure 6B shows the nucleotide sequence (Seq. ID No. 29) of a nucleic acid molecule encoding the HIV TA immunogen;

Figure 7A shows the amino acid sequence (Seq. ID No. 30) of the tat polypeptide present in the HIVAeT immunogen;

Figure 7B shows the nucleotide sequence (Seq. ID No. 31) of a nucleic acid molecule encoding the HIVAeT immunogen;

Figure 8A shows the amino acid sequence (Seq. ID No. 32) of the PPA immunogen which is in accordance with the sixth aspect of the invention; and

Figure 8B shows the nucleotide sequence (Seq. ID No. 33) of a nucleic acid molecule (in accordance with the fifth aspect of the invention) encoding the PPA immunogen.

EXAMPLES

Example 1

This example relates to an immunogen for use in a vaccine focusing on the induction of cellular immune responses mediated by a concerted action of $CD4^+$ helper and $CD8^+$ effector T lymphocytes. The immunogen, designated HIVA (Hanke & McMichael Nat. Med. 6, 951-955), was designed for a phase III efficacy trial in Nairobi, Kenya. Figure 1A is a schematic representation of several immunogens, including HIVA. HIVA is derived from the sequences of HIV-1 clade A, the predominant HIV clade in Nairobi and consists of about 73% of the gag protein fused to a string of 25 partially overlapping CTL epitopes. The gag domain of HIVA contains p24 and p17 in an order reversed to the viral gag p17-p24-p15 polyprotein. This rearrangement prevents myristylation of the N-terminus of p17, which could direct the recombinant protein to the cell membrane, thus preventing efficient degradation into peptides necessary for the major histocompatibility complex (MHC) class I presentation.

Figure 1B shows the amino acid sequence (Seq. ID No. 26) of the HIVA immunogen. Amino acids corresponding to the restriction endonuclease sites used to assemble the gene are shown in bold (GS-corresponds to *Bam* HI, GT corresponds to *Kpn*I and EF correspnds to *Eco*RI).

The amino acid sequence of the gag domain was derived from the protein database consensus sequence of HIV-1 clade A (Korber *et al*, "Human retroviruses and AIDS; a compilation and analysis of nucleic acid and amino acid sequences" 1997). In the absence of

available Kenyan strain sequences, regions without a strong amino acid clade A preference were biased towards Ugandan isolates. The HIV-1 gag protein contained not only important MHC class I-, but also class II-restricted epitopes which stimulate $CD4^+$ T helper cells.

The C-terminus of the HIVA protein was designed as a multi-CTL epitope synthetic polypeptide. The CTL epitopes included in HIVA were recognised by CTL in patients infected with HIV-1 clade A strains circulating in Kenya, were 8- to 10-amino acids long, and originate from the gag, pol, nef or env proteins (Rowland-Jones *et al*, 1998 J. Clin. Invest. 102, 1758-1765; Dorrell *et al*, 1999 J. Virol. 73, 1708-1714). Many of these epitopes are immunodominant and relatively conserved among other HIV-1 clades (Table 1) (and therefore should be able to elicit an immune response which cross-reacts with HIV viruses of clades other than clade A). They are presented by seventeen different HLA alleles, which include both frequent African alleles as well as alleles common in most ethnic populations. It has been estimated that optimally selected epitopes presented by the nine commonest HLA alleles could cover the general population irrespective of ethnic descent (Sydney *et al*, 1996 Immunol. Today 17, 261-266). Thus, given that majority of HIV-infected donors make good CTL responses to gag p17/p24, each vaccinee should have the potential to respond to at least two or three CTL epitopes present in the HIVA protein.

The HIVA synthetic polypeptide also comprised SIV gag and HIV env epitopes recognised by macaque and murine CTL, respectively, so that the quality, reproducibility and stability of the clinical batches could easily be assessed in a mouse (or macaque if necessary) potency assay. A monoclonal antibody epitope Pk (Hanke *et al*, 1992 J. Gen. Virol. 73, 653-660) was added to the C-terminus of HIVA for easy detection of the full-size protein and estimation of the level of expression. There is no reason to believe that the three non-HLA epitopes represent a health hazard for the vaccinated individuals.

Table 1. CD8+ cell epitopes included in the HIVA synthetic polypeptide polyepitope region.

Epitope[a]	MHC class I restriction	Origin	HIV clade[b]
ALKHRAYEL	HLA-A*0201	nef	a
PPIPVGEIY	HLA-B35	p24	a/B/c/D/F/G
GEIYKRWII	HLA-B8	p24	a/B/c/D/F/G
KRWIILGLNK	HLA-B*2705	p24	A/B/C/D/F/G/H
FRDYVDRFYK	HLA-B18	p24	BD(A=C/F/G/H)[c]
RDYVDRFYKTL	HLA-B44	P24	B/D(A=C/F/G/H)[c]
DRFYKTLRA	HLA-B14	p24	B/D(A=C/F/G/H)
AIFQSSMTK	HLA-A*0301, A11, A33	pol	a/B/c/D/G/H
ITLWQRPLV	HLA-A*6802	pol	a/b/C/D/F/G/H
ERYLKDQQL	HLA-B14	gp41	a/b/C/D
YLKDQQLL	HLA-A24, B8	gp41	a/b/C/D
TVYYGVPVWK	HLA-A*0301	gp120	A/B/C/D/g
RPQVPLRPMTY	HLA-B51	nef	A/b/D/E/F/G
QVPLRPMTYK	HLA-A*0301, A11	nef	A/b/D/E/F/G
VPLRPMTY	HLA-B35	nef	A/b/D/E/F/G
AVDLSHFLK	HLA-A11	nef	a/B/d/f
DLSHFLKEK	HLA-A*0301	nef	A/B/D/F
FLKEKGGL	HLA-B8	nef	A/B/C/D/E/F/G
ILKEPVHGV	HLA-A*0201	pol	A/B/C/D/G
ILKEPVHGVY	HLA-Bw62	pol	A/B/D
HPDIVIYQY	HLA-B35	pol	a
VIYQYMDDL	HLA-A*0201	pol	A/B/C/D/F/G/H
TPGPGVRYPL	HLA-B7	nef	b/c
ACTPYDINQML[d]	Mamu-A*01	p27	SIV
RGPGRAFVTI[e]	H-2D[d]	env	HIV

a - Epitopes are listed (Seq. ID Nos. 3-25, 1, 2) in the order in which they appear in the polyepitope.

b - A particular epitope sequence is present in about 50% (small letter) or 90% (capital letter) of sequenced HIV clade isolates.

c - '=' indicates that the epitopes are present in the N-terminal clade A gag domain.

d - A dominant epitope derived from SIV gag p27 flanked by Ala and Leu at its N- and C- termini, respectively, recognised by rhesus macaque CTL, which can be used for potency studies in rhesus macaques.

e - A CTL epitope presented by a murine MHC class I used for the mouse potency assay.

Since it was intended to adopt a "prime/boost" protocol, in which priming was achieved by administering nucleic acid, it was desirable to design the sequence of the nucleic acid encoding the HIVA immunogen in order to increase the expression of HIVA in human cells. Firstly, to ensure an efficient initiation of translation from the first methionine codon, the HIVA open reading frame (ORF) was preceded by a 12-nucleotide-long Kozak consensus sequence (Kozak 1987 Nucl. Acids Res. 15, 8125-8148). Secondly, the translation of the resulting mRNA was optimised by substituting most of the HIV-1-derived codons with frequently used codons in highly expressed human genes (Andre *et al*, 1998, cited above). The HIVA ORF is a part of a 1,608-base pair-long double-stranded DNA *Hin*dIII-*Xba*I fragment. (Figure 2A shows the nucleotide sequence of the *Hin*dIII-*Xba*I insert containing the HIVA ORF; Seq. ID No. 27) In Figure 2A, the endonuclease sites used to assemble the partial PCR products are included (nucleotides 1-6 = *Hin*dIII; 319-324 = *Bam* HI; 712-717 = *Kpn* I; 1135-1140 = *Eco*RI; and 1603-1608 = *Xba*I).

Plasmid DNA was prepared and treated using standard protocols (Sambrook *et al*, "Molecular Cloning. A Laboratory Manual" 2nd Edition; Cold Spring Harbor). The HIVA gene was constructed (as indicated in Figure 2B) *in vitro* in four parts. Each part was prepared by assembly from overlapping positive- and negative-strand oligodeoxynucleotides of 70-90 bases in length. The synthetic oligodeoxynucleotides were

purified using the EconoPure™ Kit (Perkin Elmer) according to the manufacturer's instructions, annealed and ligated, followed by PCR assembly. The PCR products were gel-purified after each step until fragments with the expected, unique terminal restriction endonuclease sites were obtained. These four products were cloned and sequenced, and ligated together to generate the complete HIVA gene. The HIVA gene was then inserted into the pTHr construct and MVA vaccine vector, as described below.

The pTHr vector. A vector pTHr for a direct gene transfer was designed with the aim to minimise the number of functional elements and therefore the amount of DNA required to be administered.

The construction of pTH was described previously (Hanke *et al*, 1998 Vaccine 16, 426-435). It contains an expression efficient enhancer/promoter/intron A cassette of the human cytomegalovirus (hCMV) strain AD169 genome (Whittle *et al*, 1987 Protein Eng. 1, 499-505; Bebbington 1991 Methods 2, 136-145). The promoter region is followed by the pRc/CMV (Invitrogen)-derived polylinker and polyadenylation signal of the bovine growth hormone gene. The ß-lactamase gene conferring ampicillin resistance to transformed bacteria and prokaryotic origin of double-stranded DNA replication Co1E1 are both derived from plasmid pUC19. pTH does not contain an origin for replication in mammalian cells. After insertion of the HIVA DNA into the pTH polylinker, the ß-lactamase gene fragment between the *Mlu*I and *Dra*I sites was removed and the resulting construct pTHr.HIVA (Fig. 3) was propagated in bacteria using the repressor-titration system developed by Cobra Pharmaceuticals Ltd. (Keele, UK), which selects plasmid-carrying bacteria without the need for the presence of an antibiotic-resistance gene on the plasmid (Williams *et al*, 1998 Nucl. Acids Res. 26, 2120-2124). Therefore, DNA vaccination does not introduce into the human vaccinee large numbers of copies of an antibiotic resistance gene. Construction of pTHr. HIVA is illustrated schematically in Figure 3 (CMV e/p/i = human CMV enhancer/promoter/intron A cassette; BGHpA = bovine growth hormone polyadenylation signal; ColE1 = origin of dsDNA replication in bacteria; arrow head symbols denote repressor-binding sequences).

293T cells (and chicken embryo fibroblasts [CEF] were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% foetal bovine serum (FBS; Gibco); 2mM L-glutamine and penicillin/streptomycin. Cells were cultured in a humidified incubator in 5% CO_2 at 37°C. 293T cells were transiently transfected with pTHr. HIVA using the DEAE-dextran-chloroquine method (Hanke *et al*, 1998 Vaccine 16, 426-435). Briefly, 2.5 x 10^5 293T cells were grown on coverslips in 6-well tissue culture plates overnight. The following day, cells were transfected with 5 g per well of DNA. After 48 hours, the transfected cells were fixed, their membranes were permeabilized and the SV5-P-k mAb followed by anti-murine FITC-conjugated antibodies were used to detect the expressed recombinant proteins.

A micrograph illustrating specimen results is shown in Figure 4. The Figure shows three transfected cells and one background untransfected cell (top left).

Boosting was to be achieved by administering an MVA vector expressing the HIVA immunogen. MVA is an attenuated vaccinia virus safe for clinical application, which has almost lost its ability to replicate in human cells (Mayr *et al*, 1975 Infection 105, 6-14). The use of MVA as a vaccine vehicle and its features which make it an attractive choice among the attenuated poxvirus vectors (see, e.g. Sutter *et al*, 1994 Vaccine 12, 1032-1040; and Moss *et al*, 1996 Adv. Exp. Med. Biol. 397, 7-13) have been described extensively.

The HIVA gene was ligated into plasmid pSC11, which directed the gene into the thymidine kinase locus of the parental MVA (Carroll & Moss 1995 Biotechniques 19, 352-356). Bulk stocks of the recombinant MVA were grown on primary CEF obtained from the eggs of a specific pathogen-free flock. MVA was purified by centrifugation of cytoplasmic extracts through a 36% (w/v) sucrose cushion in a Beckman SW28 rotor at 13,500 rpm for 80 minutes. Taking advantage of the co-inserted ß-galactosidase gene, the virus stock titres were determined from the number of blue plaques after incubation of the infected cell monolayers with the appropriate substrate.

Vaccine potency assay. The potencies of the DNA and MVA vectors were tested in groups of Balb/c mice taking advantage of the presence of the H-2D^d-restricted epitope (Takahashi *et al*, 1993 Int. Immunol. 5, 849-857). For the pTHr.HIVA DNA vaccine, mice were either needle-injected intramuscularly with 100 μg of DNA or immunised twice 3 weeks apart intradermally with total of 2 μg of DNA using the Dermal XR gene delivery device of PowderJect Vaccines Inc. (Madison, WI, USA). The mice were sacrificed 10 or 21 days after the last immunisation. Spleens from the immunized mice were removed and pressed individually through a cell strainer (Falcon) using a 2-ml syringe rubber plunger. The splenocytes were washed and divided into two halves. One half was frozen for tetramer analysis and the second half was suspended in 5ml of Lymphocyte medium (R10, 20 mM HEPES and 15 mM ß-mercaptoethanol) and restimulated *in vitro* by incubation with 2 μg/ml of the RGPGRAFVTI (Seq. ID No. 2) peptide in an humidified incubator in 5% CO_2 at 37°C for 5 days.

The effector cells were 2-fold diluted in U-bottom wells (96-well plate; Costar) starting with 100:1 effector to target ratio. Five thousand ^{51}Cr-labeled P815 target cells in a medium without or supplemented with 10^{-6} M peptide was then added to the effectors and incubated at 37°C for 5 hours. Spontaneous and total chromium releases were estimated from wells, in which the target cells were kept in a medium alone or with 5% Triton X-100, respectively. The percentage specific lysis was calculated as [(sample release-spontaneous release)/(total release-spontaneous release)] x 100. The spontaneous release was lower than 5% of the total c.p.m.

The results of typical assays are shown in Figures 5A-C, which are graphs of % specific lysis against Effector: Target ratio. Figure 5A shows results for splenocytes obtained from mice immunised once with 100 μg of pTHr.HIVA DNA. Figure 5B shows results from mice immunised twice intradermally with pTHr. HIVA DNA. Figure 5C shows results obtained from mice immunised intramuscularly with 10^7 pfu of MVA.HIVA. In each of Figures 5A-5C, each line represents a single mouse. Lysis of peptide-pulsed targets is denoted by filled symbols, unpulsed targets by open symbols.

Both modes of the pTHr.HIVA vector delivery were highly immunogenic and induced high cytolytic activities in all immunised animals (see Figs. 5A & B). Similarly, a single needle intramuscular injection of 10^7 plaque-forming units of MVA.HIVA elicited in all vaccinees strong peptide-specific lytic activities measured after a culture restimulation (Fig. 5C).

Example 2

Further nucleic acid constructs were prepared which encoded polyprotein immunogens based on HIVA, but comprising further HIV antigen components. These further constructs and their encoded immunogens were termed HIVTA and HIVAeT, as shown in Figure 1A. Also shown is a construct/immunogen termed PPA. The relevant DNA/amino acid sequences are shown in Figures 6 A/B-8 A/B respectively, although Figure 7A shows only that portion of amino acid sequence in HIVA eT (attributable to tat) which is additional to that in HIVTA. (Note that the sequence of the *Hin*dIII and *Xba*I sites at the 5' and 3' ends of the DNA sequences are not shown in Figures 6B, 7B and 8B). The constructs were prepared in a manner similar to that described above for HIVA.

HIVTA and HIVAeT share the same design rationale with HIVA, but additionally include the HIV-1 clade A tat sequence, expressed either as part of a fusion protein with gag and the polyepitopic synthetic polypeptide (in the case of HIVTA, the tat sequence being positioned between gag and the synthetic polypeptide), or else being present on the same construct but expressed as a separate polypeptide (in the case of HIVAeT), by virtue of the inclusion of an internal ribosome entry site (IRES).

Each of the nucleic acid molecules may be used to immunise subjects, in a manner similar to that described above in relation to HIVA DNA. Equally, the molecules may be introduced into appropriate vectors, especially MVA, again as described above in Example 1, and the resulting vector used to immunise subjects.

The significance of the genetic diversity among individual HIV isolates and its implication for vaccine design have been long debated. The predominant HIV-1 clade in Europe and

North America is clade B, which is also the most studied one. In central and Eastern Africa, the predominant circulating HIV-1 strain is clade A, while clade C is dominating Southern Africa, India and China. Generally, a clade-specific vaccine design requires a more careful consideration for the induction of nAb than for CTL. Although there are some important inter-clade differences in CTL epitopes, many epitopes are conserved across clades partially due to structure/function constraints. However, to facilitate the interpretation of efficacy studies, vaccines should attempt to match the local strains prevalent in the trial population with the view that any successful approaches can be adapted for other clades if cross-protection is not achieved.

Claims

1. An immunogen in sterile form suitable for administration to a human subject, the immunogen comprising: at least a portion of the gag protein of HIV, said gag protein being from an HIV clade or having a consensus sequence for one or more HIV clades, and comprising at least parts of p17 and p24; and a synthetic polypeptide comprising a plurality of amino acid sequences, each sequence comprising a human CTL epitope of an HIV protein, and wherein a plurality of HIV proteins are represented in the synthetic polypeptide, said CTL epitopes being selected to stimulate an immune response to one or more HIV clades of interest.

2. An immunogen according to claim 1, in which the said at least portion of gag protein and the synthetic polypeptide are present as a fusion protein.

3. An immunogen according to claim 1 or 2, in which at 55-95% of the amino acid sequence of gag protein is present in the portion of gag.

4. An immunogen according to any one of claims 1, 2 or 3, in which 65-85% of the amino acid sequence of gag protein is present in the portion of gag.

5. An immunogen according to any one of the preceding claims, in which said at least part of p17 is modified to prevent N-terminal myristylation.

6. An immunogen according to any one of the preceding claims, in which at least some of the human CTL epitopes present in the synthetic polypeptide are overlapping.

7. An immunogen according to claim 6, in which at least 50% of the human CTL epitopes present in the synthetic polypeptide are overlapping.

8. An immunogen according to any one of the preceding claims, in which at least some of the human CTL epitopes present in the synthetic polypeptide are adjacent.

9. An immunogen in according to any one of the preceding claims, in which at least some of the human CTL epitopes are separated by non-epitopic amino acid sequence.

10. An immunogen according to any one of the preceding claims, comprising at least one epitope which is recognised by one or more laboratory test mammals.

11. An immunogen according to claim 10, wherein said epitope recognised by one or more laboratory test mammals is a CTL epitope.

12. An immunogen according to any one of the preceding claims, in which the synthetic polypeptide comprises at least 10 of the human CTL epitopes identified in Table 1.

13. An immunogen according to any one of the preceding claims, in which the synthetic polypeptide comprises at least 15 of the human CTL epitopes identified in Table 1.

14. An immunogen according to any one of the preceding claims, in which the synthetic polypeptide comprises at least 20 of the human CTL epitopes identified in Table 1.

15. An immunogen according to any one of the preceding claims, in which the synthetic polypeptide comprises all 23 of the human CTL epitopes identified in Table 1.

16. An HIV immunogen for a human subject comprising at least a portion of a gag protein, said gag protein being from an HIV clade of interest or having a consensus sequence for an HIV clade of interest, and wherein said portion comprises at least parts of p17 and p24 and is modified to prevent N-terminal myristylation; and a synthetic polypeptide comprising a plurality of human CTL epitopes from a plurality of different HIV proteins, each CTL epitope having from 8 to 12 amino acids, and said CTL epitopes being selected to stimulate an immune response to the clade of interest.

17. An HIV immunogen for a human subject comprising at least a portion of a gag protein, said gag protein being from an HIV clade of interest or having a consensus sequence for an HIV clade of interest, and wherein said portion comprises at least parts of p17 and p24 and is modified to prevent N-terminal myristylation; and a synthetic polypeptide consisting essentially of a plurality of human CTL epitopes from a plurality of different HIV proteins, each CTL epitope having from 8 to 12 amino acids, and said CTL epitopes being selected to stimulate an immune response to said clade of interest; and optionally, said synthetic polypeptide having at least one immunogenic epitope recognised by a laboratory animal.

18. An immunogen according to any one of the preceding claims, comprising the amino acid sequence shown in Figure 1B.

19. An immunogen according to claim 18, consisting essentially of the amino acid sequence shown in Figure 1B.

20. An immunogen according to any one of the preceding claims, wherein said clade of interest is selected from the group consisting of HIV clade A, B, C, D, E, F, G and H.

21. An immunogen according to claim 20, wherein said clade of interest is HIV clade A.

22. An immunogen according to claim 20, wherein said clade of interest is HIV clade B.

23. An immunogen according to claim 20, wherein said clade of interest is HIV clade C.

24. An immunogen according to any one of the preceding claims, wherein said human CTL epitopes include at least one epitope from each of the HIV proteins nef, p24, p17, pol, gp41 and gp120.

25. An immunogen according to claim 24, wherein wherein said human CTL epitopes include at least one epitope from each of the HIV proteins nef, p24, p17, pol, gp41, gp120, tat, and rev.

26. An immunogen according to claim 24 wherein said human CTL epitopes include at least one epitope from each of the HIV proteins nef, p24, p17, pol, gp41, gp120, tat, rev, vpr, vpu and vif.

27. A nucleic acid molecule encoding an immunogen in accordance with any one of the preceding claims.

28. A nucleic acid according to claim 27, wherein the immunogen is encoded as a fusion protein.

29. A nucleic acid molecule according to claim 27 or 28, comprising the nucleotide sequence shown in Figure 2A.

30. A vector comprising a nucleic acid molecule in accordance with any one of claims 27, 28 or 29.

31. A particulate vector according to claim 30, further comprising an immunogen in accordance with any one of claims 1-26.

32. A method of stimulating an anti-HIV immune response in a human subject, the method comprising the steps of: preparing an immunogen in accordance with any one of claims 1-26 and/or a nucleic acid molecule in accordance with any one of claims 27-29; and administering the immunogen and/or the nucleic acid molecule to the subject.

33. A method according to claim 32, wherein the subject is primed by one or more administrations of the nucleic acid molecule; and subsequently boosted by one or more administrations of the immunogen.

34. Use of an immunogen in accordance with any one of claims 1-26 and/or a nucleic acid molecule in accordance with any one of claims 27-29, in the preparation of a medicament to prevent or treat HIV infection in a human subject.

35. A method of stimulating an anti-HIV immune response in a human subject which comprises administering one or more times an amount of a nucleic acid encoding an immunogen in accordance with any one of claims 1-26 to said subject sufficient to prime an immune response to said immunogen, and administering one or more times a modified vaccinia virus Ankara (MVA) particle encoding and/or containing an immunogen in accordance with any one of claims 1-26 to said subject in an amount sufficient to boost the immune response to common portions of said immunogens.

36. A bacterium comprising an immunogen in accordance with any one of claims 1-26, or comprising a nucleic acid molecule in accordance with any one of claims 27-29.

37. A bacterium according to claim 36 which is an attenuated pathogen suitable for administration to a human subject.

38. A nucleic acid molecule encoding a polypeptide comprising the amino acid sequence shown in Figure 8A.

39. A nucleic acid molecule encoding a polypeptide consisting essentially of the amino acid sequence shown in Figure 8A.

40. A nucleic acid molecule according to claim 38 or 39, comprising the nucleotide sequence shown in Figure 8B.

41. A polypeptide comprising the amino acid sequence shown in Figure 8A.

42. A polypeptide consisting essentially of the amino acid sequence shown in Figure 8A.

43. A particulate vector comprising the nucleic acid sequence of claim 38 or 39 and/or the polypeptide of claim 41 or 42.

44. A method of stimulating an anti-HIV immune response in a human subject, comprising the steps of: preparing a nucleic acid molecule in accordance with claim 38 or 39 and/or a polypeptide in accordance with claim 41 or 42, and administering the nucleic acid molecule and/or the polypeptide to the subject.

45. Use of a nucleic acid molecule in accordance with claim 38 or 39 and/or a polypeptide in accordance with claim 41 or 42, in the preparation of a medicament to prevent or treat HIV infection in a human subject.

46. An immunogen substantially as hereinbefore described and with reference to the accompanying drawings.

47. A method of stimulating an anti-HIV immune response in a human subject substantially as hereinbefore described and with reference to the accompanying drawings.

Fig.1A.

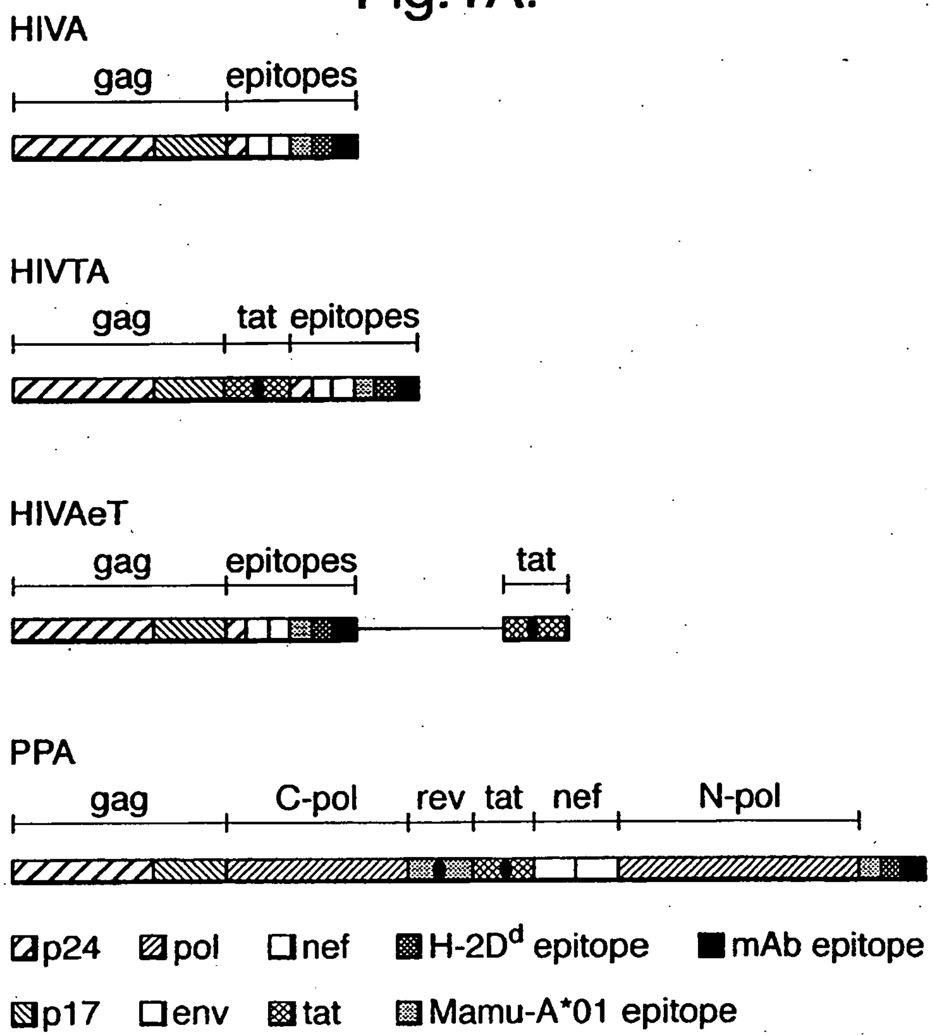

Fig.1B.

1	MPIVQNAQGQ	MHQALSPRTL	NAWVKVIEEK	AFSPEVIPMF	SALSEGATPQ	50
51	DLNMMLNIVG	GHQAAMQMLK	DTINEEAAEW	DRLHPVHAGP	IPPGQMREPR	100
101	GSDIAGTTST	LQEQIGWMTS	NPPIPVGDIY	KRWIILGLNK	IVRMYSPVSI	150
151	LDIRQGPKEP	FRDYVDRFFK	TLRAEQATQE	VKNWMTETLL	VQNANPDCKS	200
201	ILRALGPGAT	LEEMMTACQG	VGGPGHKARV	LGTGARASVL	SGGKLDAWEK	250
251	IRLRPGGKKK	YRLKHLVWAS	RELERFALNP	SLLETAEGCQ	QIMEQLQSAL	300
301	KTSEELKSLF	NTVATLYCVH	QRIDVKDTKE	ALDKIEEIQN	KSKQKTQQAA	350
351	ADTQSSSKVS	QNYALKHRAY	ELEFPPIPVG	EIYKRWIIFR	DYVDRFYKTL	400
401	RAIFQSSMTK	ITLWQRPLVE	RYLKDQQLLT	VYYGVPVWKR	PQVPLRPMTY	450
451	KAVDLSHFLK	EKGGLILKEP	VHGVYHPDIV	IYQYMDDLTP	GPGVRYPLAC	500
501	TPYDINQMLR	GPGRAFVTIP	NPLLGLD			527

Fig.2A.

HIVA DNA

AAGCTTCCCGCCGCCACCATGCCCATCGTGCAGAACGCCCAGGGCCAGATGCACCAGGCC
CTGTCCCCCCGCACCCTGAACGCCTGGGTGAAGGTGATCGAGGAGAAGGCCTTCTCCCCC
GAGGTGATCCCCATGTTCTCCGCCCTGTCCGAGGGCGCCACCCCCCAGGACCTGAACATG
ATGCTGAACATCGTGGGCGGCCACCAGGCCGCCATGCAGATGCTGAAGGACACCATCAAC
GAGGAGGCCGCCGAGTGGGACCGCCTGCACCCCGTGCACGCCGGCCCCATCCCCCCCGGC
CAGATGCGCGAGCCCCGCGGATCCGACATCGCCGGCACCACCTCCACCCTGCAGGAGCAG
ATCGGCTGGATGACCTCCAACCCCCCCATCCCCGTGGGCGACATCTACAAGCGCTGGATC
ATCCTGGGCCTGAACAAGATCGTACGCATGTACTCCCCCGTGTCCATCCTGGACATCCGC
CAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGCTTCTTCAAGACCCTGCGCGCC
GAGCAGGCCACCCAGGAGGTGAAGAACTGGATGACCGAGACCCTGCTGGTGCAGAACGCC
AACCCCGACTGCAAGTCCATCCTGCGCGCCCTGGGCCCCGGCGCCACCCTGGAGGAGATG
ATGACCGCCTGCCAGGGCGTGGGCGGCCCCGGCCACAAGGCCCGCGTGCTGGGTACCGGC
GCCCGCGCCTCCGTGCTGTCCGGCGGCAAGCTGGACGCCTGGGAGAAGATCCGCCTGCGC
CCCGGCGGCAAGAAGAAGTACCGCCTGAAGCACCTGGTGTGGGCCTCCCGCGAGCTGGAG
CGCTTCGCCCTGAACCCCTCCCTGCTGGAGACCGCCGAGGGCTGCCAGCAGATCATGGAG
CAGCTGCAGTCCGCCCTGAAGACCTCCGAGGAGCTGAAGTCCCTGTTCAACACCGTGGCC
ACCCTGTACTGCGTGCACCAGCGCATCGACGTGAAGGACACCAAGGAGGCCCTGGACAAG
ATCGAGGAGATCCAGAACAAGTCCAAGCAGAAGACCCAGCAGGCCGCCGCCGACACCCAG
TCCTCCTCCAAGGTGTCCCAGAACTACGCCCTGAAGCACCGCGCCTACGAGCTGGAATTC
CCTCCAATTCCTGTCGGGGAGATTTATAAACGGTGGATCATTTTTAGGATTATGTCGAT
AGGTTTTATAAAACGCTCAGGGCCATCTTCCAGTCCTCCATGACCAAGATCACCCTGTGG
CAGCGCCCCCTGGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGACCGTGTACTACGGC
GTGCCCGTGTGGAAGCGCCCCCAGGTGCCCCTGCGCCCCATGACCTACAAGGCCGTGGAC
CTGTCCCACTTCCTGAAGGAGAAGGGCGGCCTGATCCTGAAGGAGCCCGTGCACGGCGTG
TACCACCCCGACATCGTGATCTACCAGTACATGGACGACCTGACCCCCGGCCCCGGCGTG
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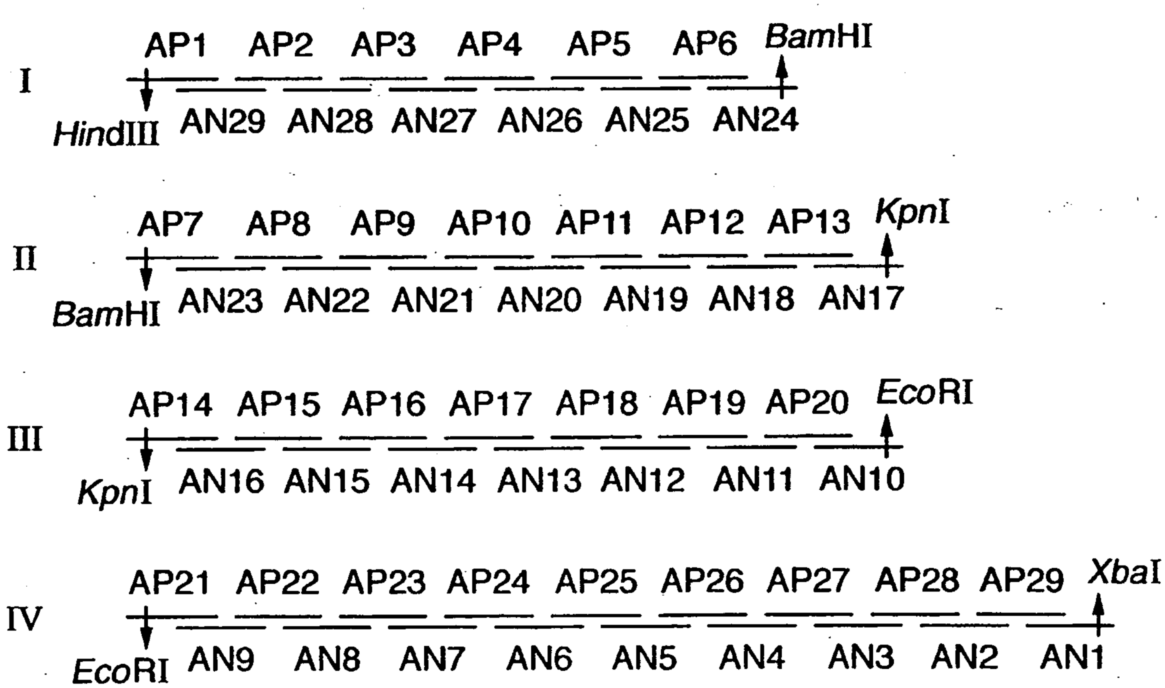
Fig.2B.


I
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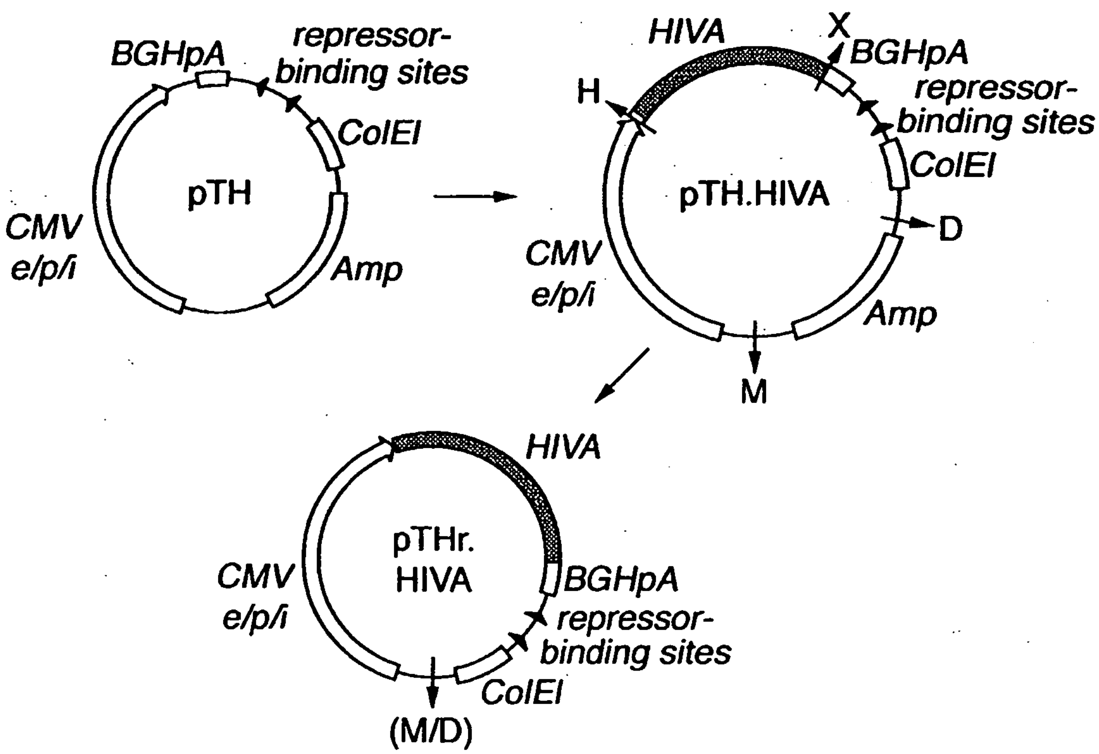
HindIII AN29 AN28 AN27 AN26 AN25 AN24
II
AP7 AP8 AP9 AP10 AP11 AP12 AP13 KpnI
BamHI AN23 AN22 AN21 AN20 AN19 AN18 AN17
III
AP14 AP15 AP16 AP17 AP18 AP19 AP20 EcoRI
KpnI AN16 AN15 AN14 AN13 AN12 AN11 AN10
IV
AP21 AP22 AP23 AP24 AP25 AP26 AP27 AP28 AP29 XbaI
EcoRI AN9 AN8 AN7 AN6 AN5 AN4 AN3 AN2 AN1

Fig.3.


BGHpA
repressor-
binding sites
ColEI
pTH
CMV
e/p/i
Amp
HIVA
X
H
BGHpA
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binding sites
ColEI
pTH.HIVA
D
CMV
e/p/i
Amp
M
HIVA
pTHr.
HIVA
CMV
e/p/i
BGHpA
repressor-
binding sites
ColEI
(M/D)

Fig.4.

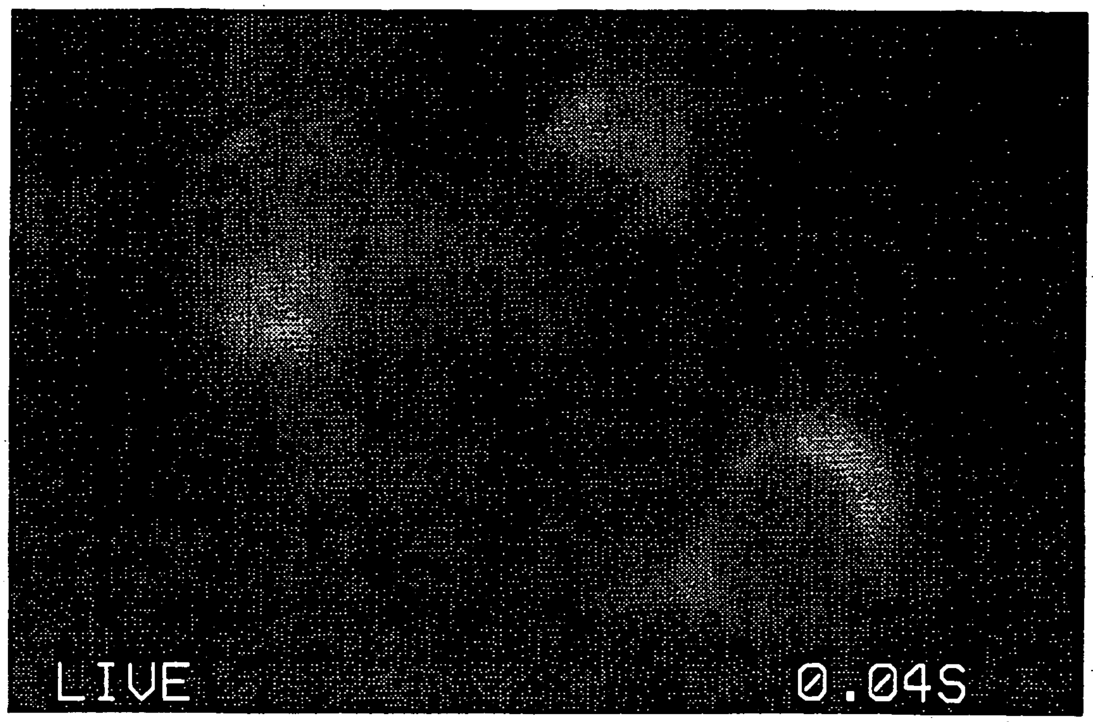

Fig.5A.

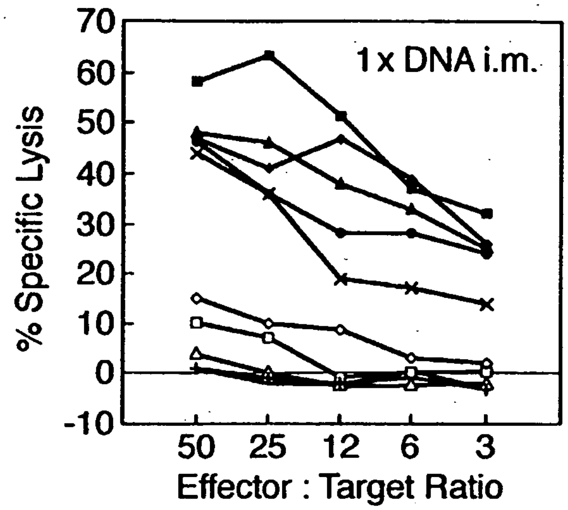

Fig.5B.

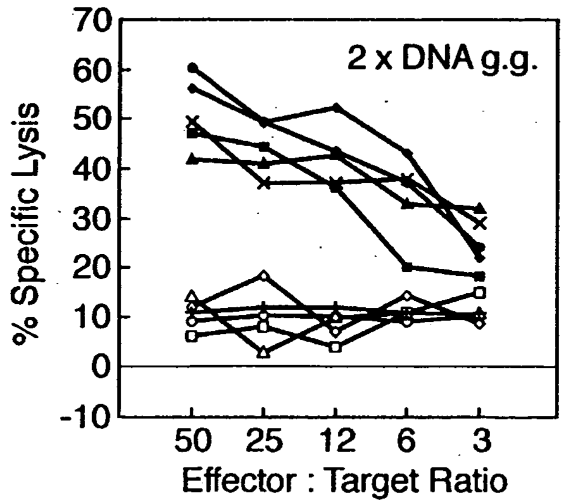

Fig.5C.

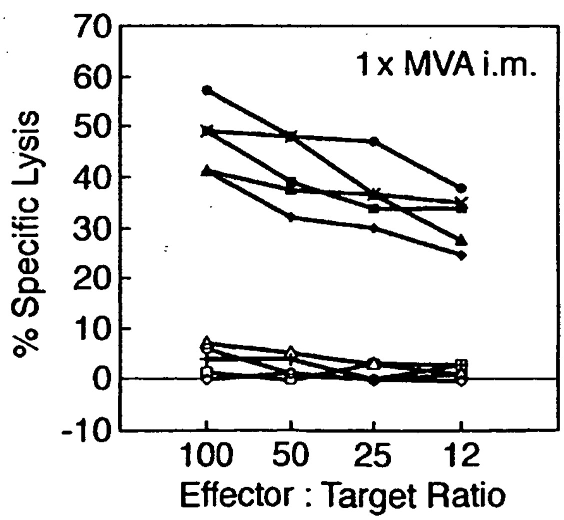

Fig.6B.

HIVTA DNA

CCCGCCGCCACCATGCCCATCGTGCAGAACGCCCAGGGCCAGATGCACCAGGCCCTGTCC
CCCCGCACCCTGAACGCCTGGGTGAAGGTGATCGAGGAGAAGGCCTTCTCCCCCGAGGTG
ATCCCCATGTTCTCCGCCCTGTCCGAGGGCGCCACCCCCCAGGACCTGAACATGATGCTG
AACATCGTGGGCGGCCACCAGGCCGCCATGCAGATGCTGAAGGACACCATCAACGAGGAG
GCCGCCGAGTGGGACCGCCTGCACCCCGTGCACGCCGGCCCCATCCCCCCCGGCCAGATG
CGCGAGCCCCGCGGATCCGACATCGCCGGCACCACCTCCACCCTGCAGGAGCAGATCGGC
TGGATGACCTCCAACCCCCCCATCCCCGTGGGCGACATCTACAAGCGCTGGATCATCCTG
GGCCTGAACAAGATCGTGCGCATGTACTCCCCCGTGTCCATCCTGGACATCCGCCAGGGC
CCCAAGGAGCCCTTCCGCGACTACGTGGACCGCTTCTTCAAGACCCTGCGCGCCGAGCAG
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GCCTCCGTGCTGTCCGGCGGCAAGCTGGACGCCTGGGAGAAGATCCGCCTGCGCCCCGGC
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GGCCCCGGCGTGCGCTACCCCCTGGCCTGCACCCCCTACGACATCAACCAGATGCTGCGC
GGCCCCGGCCGCGCCTTCGTGACCATCCCCAACCCCCTGCTGGGCCTGGACTGA

Fig.6A.

HIVTA protein

MPIVQNAQGQMHQALSPRTLNAWVKVIEEKAFSPEVIPMFSALSEGATPQDLNM
MLNIVGGHQAAMQMLKDTINEEAAEWDRLHPVHAGPIPPGQMREPRGSDIAGT
TSTLQEQIGWMTSNPPIPVGDIYKRWIILGLNKIVRMYSPVSILDIRQGPKEPFRDY
VDRFFKTLRAEQATQEVKNWMTETLLVQNANPDCKSILRALGPGATLEEMMTA
CQGVGGPGHKARVLGTGARASVLSGGKLDAWEKIRLRPGGKKKYRLKHLVWA
SRELERFALNPSLLETAEGCQQIMEQLQSALKTSEELKSLFNTVATLYCVHQRIDV
KDTKEALDKIEEIQNKSKQKTQQAADTQSSSKVSQNYALKHRAYELEFMATTM
DPVDPNLEPWNHPGSQPTTPGSKCYCKVCCYHCPVCFLNKGLGISYGRKKRRQR
RGTPQSNKDHQNPIPKQPIPQTQGISTGPKESKKKVESKTETDPEEFPPIPVGEIYK
RWIIFRDYVDRFYKTLRAIFQSSMTKITLWQRPLVERYLKDQQLLTVYYGVPVW
KRPQVPLRPMTYKAVDLSHFLKEKGGLILKEPVHGVYHPDIVIYQYMDDLTPGP
GVRYPLACTPYDINQMLRGPGRAFVTIPNPLLGLD

Fig.7B.

HIVAeT DNA

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GCCACCCAGGAGGTGAAGAACTGGATGACCGAGACCCTGCTGGTGCAGAACGCCAACCCC
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GCCCTGAACCCCTCCCTGCTGGAGACCGCCGAGGGCTGCCAGCAGATCATGGAGCAGCTG
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AAGCAGCCCATCCCCCAGACCCAGGGCATCTCCACCGGCCCCAAGGAGTCCAAGAAGAAG
GTGGAGTCCAAGACCGAGACCGACCCCGAGTAA

Fig.7A.

HIVAeT protein – TAT (HIVA is the same as above)
MATTMDPVDPNLEPWNHPGSQPTTPGSKCYCKVCCYHCPVCFLNKGLGISYGR
KKRRQRRGTPQSNKDHQNPIPKQPIPQTQGISTGPKESKKKVESKTETDPE

Fig.8A.

PPA protein
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VDRFFKTLRAEQATQEVKNWMTETLLVQNANPDCKSILRALGPGATLEEMMTA
CQGVGGPGHKARVLGTGARASVLSGGKLDAWEKIRLRPGGKKKYRLKHLVWA
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HQKEPPFLWMGYELHPDKWTVQPIELPEKDSWTVNDIQKLVGKLNWASQIYAC
TPYDINQMLRGPGRAFVTIPNPLLGLD

Fig.8B.

PPA DNA

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Fig.8B(Cont.)

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International Aids Vaccine Initiative
University of Nairobi

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Pro Gln Asp Leu Asn Met Met Leu Asn Ile Val Gly Gly His Gln Ala
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Ala Met Gln Met Leu Lys Asp Thr Ile Asn Glu Glu Ala Ala Glu Trp
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Asp Arg Leu His Pro Val His Ala Gly Pro Ile Pro Pro Gly Gln Met
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Glu Gln Ile Gly Trp Met Thr Ser Asn Pro Pro Ile Pro Val Gly Asp
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Ser Gly Gly Lys Leu Asp Ala Trp Glu Lys Ile Arg Leu Arg Pro Gly
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Glu Ile Gln Asn Lys Ser Lys Gln Lys Thr Gln Gln Ala Ala Ala Asp
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Ala Tyr Glu Leu Glu Phe Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys
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Arg Trp Ile Ile Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu
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Arg Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Thr Leu Trp Gln Arg
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Leu Ile Leu Lys Glu Pro Val His Gly Val Tyr His Pro Asp Ile Val
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atgaccgcct gccagggcgt gggcggcccc ggccacaagg cccgcgtgct gggtaccggc 720
gcccgcgcct ccgtgctgtc cggcggcaag ctggacgcct gggagaagat ccgcctgcgc 780
cccggcggca agaagaagta ccgcctgaag cacctggtgt gggcctcccg cgagctggag 840
cgcttcgccc tgaacccctc cctgctggag accgccgagg gctgccagca gatcatggag 900
cagctgcagt ccgccctgaa gacctccgag gagctgaagt ccctgttcaa caccgtggcc 960

accctgtact gcgtgcacca gcgcatcgac gtgaaggaca ccaaggaggc cctggacaag 1020
atcgaggaga tccagaacaa gtccaagcag aagacccagc aggccgccgc cgacacccag 1080
tcctcctcca aggtgtccca gaactacgcc ctgaagcacc gcgcctacga gctggaattc 1140
cctccaattc ctgtcgggga gatttataaa cggtggatca tttttaggga ttatgtcgat 1200
aggttttata aaacgctcag ggccatcttc cagtcctcca tgaccaagat caccctgtgg 1260
cagcgccccc tggtggagcg ctacctgaag gaccagcagc tgctgaccgt gtactacggc 1320
gtgcccgtgt ggaagcgccc ccaggtgccc ctgcgcccca tgacctacaa ggccgtggac 1380
ctgtcccact tcctgaagga gaagggcggc ctgatcctga aggagcccgt gcacggcgtg 1440
taccaccccg acatcgtgat ctaccagtac atggacgacc tgacccccgg ccccggcgtg 1500
cgctaccccc tggcctgcac cccctacgac atcaaccaga tgctgcgcgg ccccggccgc 1560
gccttcgtga ccatccccaa ccccctgctg ggcctggact gatctaga 1608

<210> 28
<211> 633
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Chimeric
polypeptide

<400> 28
Met Pro Ile Val Gln Asn Ala Gln Gly Gln Met His Gln Ala Leu Ser
1 5 10 15

Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lys Ala Phe
20 25 30

Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr
35 40 45

Pro Gln Asp Leu Asn Met Met Leu Asn Ile Val Gly Gly His Gln Ala
50 55 60

Ala Met Gln Met Leu Lys Asp Thr Ile Asn Glu Glu Ala Ala Glu Trp
65 70 75 80

Asp Arg Leu His Pro Val His Ala Gly Pro Ile Pro Pro Gly Gln Met
85 90 95

Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln
100 105 110

Glu Gln Ile Gly Trp Met Thr Ser Asn Pro Pro Ile Pro Val Gly Asp
115 120 125

Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met
130 135 140

Tyr Ser Pro Val Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro
145 150 155 160

Phe Arg Asp Tyr Val Asp Arg Phe Phe Lys Thr Leu Arg Ala Glu Gln
165 170 175

Ala Thr Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln
180 185 190

Asn Ala Asn Pro Asp Cys Lys Ser Ile Leu Arg Ala Leu Gly Pro Gly
195 200 205

Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro
210 215 220

Gly His Lys Ala Arg Val Leu Gly Thr Gly Ala Arg Ala Ser Val Leu
225 230 235 240

Ser Gly Gly Lys Leu Asp Ala Trp Glu Lys Ile Arg Leu Arg Pro Gly
245 250 255

Gly Lys Lys Lys Tyr Arg Leu Lys His Leu Val Trp Ala Ser Arg Glu
260 265 270

Leu Glu Arg Phe Ala Leu Asn Pro Ser Leu Leu Glu Thr Ala Glu Gly
275 280 285

Cys Gln Gln Ile Met Glu Gln Leu Gln Ser Ala Leu Lys Thr Ser Glu
290 295 300

Glu Leu Lys Ser Leu Phe Asn Thr Val Ala Thr Leu Tyr Cys Val His
305 310 315 320

Gln Arg Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Asp Lys Ile Glu
325 330 335

Glu Ile Gln Asn Lys Ser Lys Gln Lys Thr Gln Gln Ala Ala Ala Asp
340 345 350

Thr Gln Ser Ser Ser Lys Val Ser Gln Asn Tyr Ala Leu Lys His Arg
355 360 365

Ala Tyr Glu Leu Glu Phe Met Ala Thr Thr Met Asp Pro Val Asp Pro
370 375 380

Asn Leu Glu Pro Trp Asn His Pro Gly Ser Gln Pro Thr Thr Pro Gly
385 390 395 400

Ser Lys Cys Tyr Cys Lys Val Cys Cys Tyr His Cys Pro Val Cys Phe
405 410 415

Leu Asn Lys Gly Leu Gly Ile Ser Tyr Gly Arg Lys Lys Arg Arg Gln
420 425 430

Arg Arg Gly Thr Pro Gln Ser Asn Lys Asp His Gln Asn Pro Ile Pro
435 440 445

Lys Gln Pro Ile Pro Gln Thr Gln Gly Ile Ser Thr Gly Pro Lys Glu
450 455 460

Ser Lys Lys Lys Val Glu Ser Lys Thr Glu Thr Asp Pro Glu Glu Phe
465 470 475 480

Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Phe Arg
485 490 495

Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Ile Phe Gln Ser
500 505 510

Ser Met Thr Lys Ile Thr Leu Trp Gln Arg Pro Leu Val Glu Arg Tyr
515 520 525

Leu Lys Asp Gln Gln Leu Leu Thr Val Tyr Tyr Gly Val Pro Val Trp
530 535 540

Lys Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Ala Val Asp
545 550 555 560

Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Ile Leu Lys Glu Pro
565 570 575

Val His Gly Val Tyr His Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp
580 585 590

Asp Leu Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Ala Cys Thr Pro
595 600 605

Tyr Asp Ile Asn Gln Met Leu Arg Gly Pro Gly Arg Ala Phe Val Thr
610 615 620

Ile Pro Asn Pro Leu Leu Gly Leu Asp
625 630

<210> 29
<211> 1914
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Chimeric polynucleotide

<400> 29
cccgccgcca ccatgcccat cgtgcagaac gcccagggcc agatgcacca ggccctgtcc 60
ccccgcaccc tgaacgcctg ggtgaaggtg atcgaggaga aggccttctc cccсgaggtg 120
atccccatgt tctccgccct gtccgagggc gccacccccc aggacctgaa catgatgctg 180
aacatcgtgg gcggccacca ggccgccatg cagatgctga aggacaccat caacgaggag 240
gccgccgagt gggaccgcct gcaccccgtg cacgccggcc ccatcccccc cggccagatg 300
cgcgagcccc gcggatccga catcgccggc accacctcca ccctgcagga gcagatcggc 360
tggatgacct ccaacccccc catccccgtg ggcgacatct acaagcgctg gatcatcctg 420
ggcctgaaca agatcgtgcg catgtactcc cccgtgtcca tcctggacat ccgccagggc 480
cccaaggagc ccttccgcga ctacgtggac cgcttcttca agaccctgcg cgccgagcag 540
gccacccagg aggtgaagaa ctggatgacc gagaccctgc tggtgcagaa cgccaacccc 600
gactgcaagt ccatcctgcg cgccctgggc cccggcgcca ccctggagga gatgatgacc 660
gcctgccagg gcgtgggcgg ccccggccac aaggcccgcg tgctgggtac cggcgcccgc 720
gcctccgtgc tgtccggcgg caagctggac gcctgggaga agatccgcct gcgccccggc 780
ggcaagaaga agtaccgcct gaagcacctg gtgtgggcct cccgcgagct ggagcgcttc 840
gccctgaacc cctccctgct ggagaccgcc gagggctgcc agcagatcat ggagcagctg 900
cagtccgccc tgaagacctc cgaggagctg aagtccctgt tcaacaccgt ggccaccctg 960
tactgcgtgc accagcgcat cgacgtgaag gacaccaagg aggccctgga caagatcgag 1020
gagatccaga acaagtccaa gcagaagacc cagcaggccg ccgccgacac ccagtcctcc 1080
tccaaggtgt cccagaacta cgccctgaag caccgcgcct acgagctgga attcatggcc 1140
acaaccatgg accccgtgga ccccaacctg gagccctgga accaccccgg ctcccagccc 1200
accacccccg gctccaagtg ctactgcaag gtgtgctgct accactgccc cgtgtgcttc 1260
ctgaacaagg gcctgggcat ctcctacggc cgcaagaagc gccgccagcg ccgcggcacc 1320
ccccagtcca acaaggacca ccagaacccc atccccaagc agcccatccc ccagacccag 1380
ggcatctcca ccggtcccaa ggagtccaag aagaaggtgg agtccaagac cgagaccgac 1440
cccgaggaat tccctccaat tcctgtcggg gagatttata aacggtggat catttttagg 1500

gattatgtcg ataggtttta taaaacgctc agggccatct tccagtcctc catgaccaag 1560
atcaccctgt ggcagcgccc cctggtggag cgctacctga aggaccagca gctgctgacc 1620
gtgtactacg gcgtgcccgt gtggaagcgc ccccaggtgc ccctgcgccc catgacctac 1680
aaggccgtgg acctgtccca cttcctgaag gagaagggcg gcctgatcct gaaggagccc 1740
gtgcacggcg tgtaccaccc cgacatcgtg atctaccagt acatggacga cctgaccccc 1800
ggccccggcg tgcgctaccc cctggcctgc accccctacg acatcaacca gatgctgcgc 1860
ggccccggcc gcgccttcgt gaccatcccc aaccccctgc tgggcctgga ctga 1914

<210> 30
<211> 104
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Chimeric
polypeptide

<400> 30
Met Ala Thr Thr Met Asp Pro Val Asp Pro Asn Leu Glu Pro Trp Asn
1 5 10 15

His Pro Gly Ser Gln Pro Thr Thr Pro Gly Ser Lys Cys Tyr Cys Lys
20 25 30

Val Cys Cys Tyr His Cys Pro Val Cys Phe Leu Asn Lys Gly Leu Gly
35 40 45

Ile Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Gly Thr Pro Gln
50 55 60

Ser Asn Lys Asp His Gln Asn Pro Ile Pro Lys Gln Pro Ile Pro Gln
65 70 75 80

Thr Gln Gly Ile Ser Thr Gly Pro Lys Glu Ser Lys Lys Lys Val Glu
85 90 95

Ser Lys Thr Glu Thr Asp Pro Glu
100

<210> 31
<211> 2493
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Chimeric
polynucleotide

<400> 31
cccgccgcca ccatgcccat cgtgcagaac gcccagggcc agatgcacca ggccctgtcc 60
ccccgcaccc tgaacgcctg ggtgaaggtg atcgaggaga aggccttctc ccccgaggtg 120
atccccatgt tctccgccct gtccgagggc gccacccccc aggacctgaa catgatgctg 180
aacatcgtgg gcggccacca ggccgccatg cagatgctga aggacaccat caacgaggag 240
gccgccgagt gggaccgcct gcaccccgtg cacgccggcc ccatcccccc cggccagatg 300
cgcgagcccc gcggatccga catcgccggc accacctcca ccctgcagga gcagatcggc 360
tggatgacct ccaacccccc catccccgtg ggcgacatct acaagcgctg gatcatcctg 420
ggcctgaaca agatcgtgcg catgtactcc cccgtgtcca tcctggacat ccgccagggc 480
cccaaggagc ccttccgcga ctacgtggac cgcttcttca agaccctgcg cgccgagcag 540
gccacccagg aggtgaagaa ctggatgacc gagaccctgc tggtgcagaa cgccaacccc 600
gactgcaagt ccatcctgcg cgccctgggc cccggcgcca ccctggagga gatgatgacc 660
gcctgccagg gcgtgggcgg ccccggccac aaggcccgcg tgctgggtac cggcgcccgc 720
gcctccgtgc tgtccggcgg caagctggac gcctgggaga agatccgcct gcgccccggc 780
ggcaagaaga agtaccgcct gaagcacctg gtgtgggcct cccgcgagct ggagcgcttc 840
gccctgaacc cctccctgct ggagaccgcc gagggctgcc agcagatcat ggagcagctg 900
cagtccgccc tgaagacctc cgaggagctg aagtccctgt tcaacaccgt ggccaccctg 960
tactgcgtgc accagcgcat cgacgtgaag gacaccaagg aggccctgga caagatcgag 1020
gagatccaga acaagtccaa gcagaagacc cagcaggccg ccgccgacac ccagtcctcc 1080
tccaaggtgt cccagaacta cgccctgaag caccgcgcct acgagctgga attccctcca 1140
attcctgtcg ggagatttta taaacggtgg atcattttta gggattatgt cgataggttt 1200
tataaaacgc tcagggccat cttccagtcc tccatgacca agatcaccct gtggcagcgc 1260
cccctggtgg agcgctacct gaaggaccag cagctgctga ccgtgtacta cggcgtgccc 1320
gtgtggaagc gcccccaggt gcccctgcgc cccatgacct acaaggccgt ggacctgtcc 1380
cacttcctga aggagaaggg cggcctgatc ctgaaggagc ccgtgcacgg cgtgtaccac 1440
cccgacatcg tgatctacca gtacatggac gacctgaccc ccggccccgg cgtgcgctac 1500
cccctggcct gcacccccta cgacatcaac cagatgctgc gcggccccgg ccgcgccttc 1560
gtgaccatcc ccaacccccct gctgggcctg gactgagcgg ccgcccctct ccctcccccc 1620
cccctaacgt tactggccga agccgcttgg aataaggccg gtgtgcgttt gtctatatgt 1680
tattttccac catattgccg tcttttggca atgtgagggc ccggaaacct ggccctgtct 1740
tcttgacgag cattcctagg ggtctttccc ctctcgccaa aggaatgcaa ggtctgttga 1800
atgtcgtgaa ggaagcagtt cctctggaag cttcttgaag acaaacaacg tctgtagcga 1860
ccctttgcag gcagcggaac cccccacctg gcgacaggtg cctctgcggc caaaagccac 1920
gtgtataaga tacacctgca aaggcggcac aaccccagtg ccacgttgtg agttggatag 1980
ttgtggaaag agtcaaatgg ctctcctcaa gcgtattcaa caaggggctg aaggatgccc 2040
agaaggtacc ccattgtatg ggatctgatc tggggcctcg gtgcacatgc tttacatgtg 2100
tttagtcgag gttaaaaaaa cgtctaggcc ccccgaacca cggggacgtg gttttccttt 2160
gaaaaacacg atgataatat ggccacaacc atggaccccg tggaccccaa cctggagccc 2220
tggaaccacc ccggctccca gcccaccacc cccggctcca agtgctactg caaggtgtgc 2280
tgctaccact gccccgtgtg cttcctgaac aagggcctgg gcatctccta cggccgcaag 2340
aagcgccgcc agcgccgcgg caccccccag tccaacaagg accaccagaa ccccatcccc 2400

aagcagccca tcccccagac ccagggcatc tccaccggcc ccaaggagtc caagaagaag 2460
gtggagtcca agaccgagac cgaccccgag taa 2493

<210> 32
<211> 1445
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: Chimeric
polypeptide

<400> 32
Met Pro Ile Val Gln Asn Ala Gln Gly Gln Met His Gln Ala Leu Ser
1 5 10 15

Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lys Ala Phe
20 25 30

Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr
35 40 45

Pro Gln Asp Leu Asn Met Met Leu Asn Ile Val Gly Gly His Gln Ala
50 55 60

Ala Met Gln Met Leu Lys Asp Thr Ile Asn Glu Glu Ala Ala Glu Trp
65 70 75 80

Asp Arg Leu His Pro Val His Ala Gly Pro Ile Pro Pro Gly Gln Met
85 90 95

Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln
100 105 110

Glu Gln Ile Gly Trp Met Thr Ser Asn Pro Pro Ile Pro Val Gly Asp
115 120 125

Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met
130 135 140

Tyr Ser Pro Val Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro
145 150 155 160

Phe Arg Asp Tyr Val Asp Arg Phe Phe Lys Thr Leu Arg Ala Glu Gln
165 170 175

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Ala Thr Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln
            180                 185                 190

Asn Ala Asn Pro Asp Cys Lys Ser Ile Leu Arg Ala Leu Gly Pro Gly
        195                 200                 205

Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro
    210                 215                 220

Gly His Lys Ala Arg Val Leu Gly Thr Gly Ala Arg Ala Ser Val Leu
225                 230                 235                 240

Ser Gly Gly Lys Leu Asp Ala Trp Glu Lys Ile Arg Leu Arg Pro Gly
                245                 250                 255

Gly Lys Lys Lys Tyr Arg Leu Lys His Leu Val Trp Ala Ser Arg Glu
            260                 265                 270

Leu Glu Arg Phe Ala Leu Asn Pro Ser Leu Leu Glu Thr Ala Glu Gly
        275                 280                 285

Cys Gln Gln Ile Met Glu Gln Leu Gln Ser Ala Leu Lys Thr Ser Glu
    290                 295                 300

Glu Leu Lys Ser Leu Phe Asn Thr Val Ala Thr Leu Tyr Cys Val His
305                 310                 315                 320

Gln Arg Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Asp Lys Ile Glu
                325                 330                 335

Glu Ile Gln Asn Lys Ser Lys Gln Lys Thr Gln Gln Ala Ala Ala Asp
            340                 345                 350

Thr Gln Ser Ser Ser Lys Val Ser Gln Asn Tyr Ala Leu Lys His Arg
        355                 360                 365

Ala Tyr Glu Leu Glu Phe Gly Ile Lys Val Lys Gln Leu Cys Lys Leu
    370                 375                 380

Leu Arg Gly Ala Lys Ala Leu Thr Asp Ile Val Thr Leu Thr Glu Glu
385                 390                 395                 400

Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Asp Pro Val
                405                 410                 415
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His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln
420 425 430

Lys Gln Gly Gln Asp Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe
435 440 445

Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Lys Arg Ser Ala Gln Thr
450 455 460

Asn Asp Val Lys Gln Leu Ala Glu Val Val Gln Lys Val Val Met Glu
465 470 475 480

Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Arg Leu Pro Ile Gln
485 490 495

Lys Glu Thr Trp Glu Thr Trp Trp Met Asp Tyr Trp Gln Ala Thr Trp
500 505 510

Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp
515 520 525

Tyr Gln Leu Glu Lys Asp Pro Ile Ala Gly Ala Glu Thr Phe Tyr Val
530 535 540

Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val
545 550 555 560

Thr Asp Arg Gly Arg Gln Lys Val Val Ser Leu Thr Glu Thr Thr Asn
565 570 575

Gln Lys Thr Glu Leu His Val Ile His Leu Ala Leu Gln Asp Ser Gly
580 585 590

Ser Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile
595 600 605

Gln Ala Gln Pro Asp Arg Ser Asp Pro Val Asp Pro Asn Leu Glu Pro
610 615 620

Trp Asn His Pro Gly Ser Gln Pro Thr Thr Pro Gly Ser Lys Cys Tyr
625 630 635 640

Cys Lys Val Cys Cys Tyr His Cys Pro Val Cys Phe Leu Asn Lys Gly
645 650 655

Leu Gly Ile Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Gly Thr
660 665 670

Pro Gln Ser Asn Lys Asp His Gln Asn Pro Ile Pro Lys Gln Pro Ile
675 680 685

Pro Gln Thr Gln Gly Ile Ser Thr Gly Pro Lys Glu Ser Lys Lys Lys
690 695 700

Val Glu Ser Lys Thr Glu Thr Asp Pro Glu Asp Ala Gly Arg Ser Gly
705 710 715 720

Asn Ser Asp Glu Glu Leu Leu Lys Ala Ile Arg Ile Ile Lys Ile Leu
725 730 735

Tyr Gln Ser Asn Pro Tyr Pro Lys Pro Lys Gly Ser Arg Gln Ala Arg
740 745 750

Lys Asn Arg Arg Arg Arg Trp Arg Ala Gly Gln Arg Gln Ile Asp Ser
755 760 765

Leu Ser Glu Arg Ile Leu Ser Thr Cys Leu Gly Arg Pro Ala Glu Pro
770 775 780

Val Pro Leu Gln Leu Pro Pro Leu Glu Leu Asp Cys Ser Glu Asp Cys
785 790 795 800

Gly Thr Ser Gly Thr Gln Gln Ser Gln Gly Ala Glu Thr Gly Val Gly
805 810 815

Arg Pro Gln Val Ser Val Glu Ser Ser Ala Val Leu Gly Ser Gly Thr
820 825 830

Lys Glu Gly Thr Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
835 840 845

Lys Ala Ala Phe Asp Leu Ser Phe Phe Leu Lys Glu Lys Gly Gly Leu
850 855 860

Asp Gly Leu Ile Tyr Ser Lys Lys Arg Gln Glu Ile Leu Asp Leu Trp
865 870 875 880

Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
885 890 895

Gly Pro Gly Ile Arg Tyr Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
900 905 910

Val Pro Val Asp Pro Asp Glu Val Glu Glu Ala Thr Gly Gly Glu Asn
915 920 925

Asn Ser Leu Leu His Pro Ile Cys Gln His Gly Met Asp Asp Glu Glu
930 935 940

Lys Glu Thr Leu Arg Trp Lys Phe Asp Ser Ser Leu Ala Leu Lys His
945 950 955 960

Arg Ala Arg Glu Leu His Pro Glu Ser Tyr Lys Asp Cys Pro Gln Ile
965 970 975

Thr Leu Trp Gln Arg Pro Leu Val Thr Lys Ile Gly Gly Gln Lys Thr
980 985 990

Arg Gly Gly Lys Trp Ser Lys Ser Ser Ile Val Gly Trp Pro Glu Val
995 1000 1005

Arg Glu Arg Ile Arg Gln Thr Pro Thr Ala Ala Arg Glu Arg Thr Arg
1010 1015 1020

Gln Ala Pro Thr Ala Ala Lys Val Gly Ala Val Ser Gln Asp Leu Asp
1025 1030 1035 1040

Lys His Gly Ala Val Ser Ser Asn Val Asn His Pro Ser Cys Ala Trp
1045 1050 1055

Leu Glu Ala Gln Glu Glu Glu Glu Val Gly Phe Pro Glu Leu Leu Asp
1060 1065 1070

Thr Gly Ala Asp Asp Thr Val Leu Glu Asp Ile Asn Leu Pro Gly Lys
1075 1080 1085

Trp Lys Pro Lys Met Ile Gly Gly Ile Gly Gly Leu Ile Lys Val Lys
1090 1095 1100

Gln Tyr Asp Gln Ile Leu Ile Glu Ile Cys Gly Lys Lys Ala Ile Gly
1105 1110 1115 1120

Thr Val Leu Val Gly Pro Thr Pro Val Asn Ile Ile Gly Arg Asn Met
1125 1130 1135

Leu Thr Gln Ile Gly Cys Thr Leu Asn Phe Pro Ile Ser Pro Ile Glu
1140 1145 1150

Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys
1155 1160 1165

Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Thr Glu Ile Cys
1170 1175 1180

Ala Asp Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn
1185 1190 1195 1200

Pro Tyr Asn Thr Pro Ile Phe Ala Ile Lys Lys Lys Gln Ser Thr Lys
1205 1210 1215

Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp
1220 1225 1230

Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys
1235 1240 1245

Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val
1250 1255 1260

Pro Leu Asp Glu Ser Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser
1265 1270 1275 1280

Thr Asn Asn Glu Thr Pro Gly Val Arg Tyr Gln Tyr Asn Val Leu Pro
1285 1290 1295

Gln Gly Trp Lys Gly Ser Pro Ile Phe Gln Ser Ser Met Thr Lys Ile
1300 1305 1310

Leu Glu Pro Phe Arg Ser Lys Asn Pro Asp Ile Val Ile Tyr Gln Tyr
1315 1320 1325

Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg
1330 1335 1340

Thr Lys Ile Glu Glu Leu Arg Ala His Leu Leu Ser Trp Gly Phe Ile
1345 1350 1355 1360

Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly
1365 1370 1375

Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Glu Leu Pro
1380 1385 1390

Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys
1395 1400 1405

Leu Asn Trp Ala Ser Gln Ile Tyr Ala Cys Thr Pro Tyr Asp Ile Asn
1410 1415 1420

Gln Met Leu Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Pro Asn Pro
1425 1430 1435 1440

Leu Leu Gly Leu Asp
1445

<210> 33
<211> 4350
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Chimeric polynucleotide

<400> 33
cccgccgcca ccatgcccat cgtgcagaac gcccagggcc agatgcacca ggccctgtcc 60
ccccgcaccc tgaacgcctg ggtgaaggtg atcgaggaga aggccttctc ccccgaggtg 120
atccccatgt tctccgccct gtccgagggc gccacccccc aggacctgaa catgatgctg 180
aacatcgtgg gcggccacca ggccgccatg cagatgctga aggacaccat caacgaggag 240
gccgccgagt gggaccgcct gcaccccgtg cacgccggcc ccatcccccc cggccagatg 300
cgcgagcccc gcggatccga catcgccggc accacctcca ccctgcagga gcagatcggc 360
tggatgacct ccaacccccc catccccgtg ggcgacatct acaagcgctg gatcatcctg 420
ggcctgaaca agatcgtgcg catgtactcc cccgtgtcca tcctggacat ccgccagggc 480
cccaaggagc ccttccgcga ctacgtggac cgcttcttca agaccctgcg cgccgagcag 540
gccacccagg aggtgaagaa ctggatgacc gagaccctgc tggtgcagaa cgccaacccc 600
gactgcaagt ccatcctgcg cgccctgggc cccggcgcca ccctggagga gatgatgacc 660
gcctgccagg gcgtgggcgg ccccggccac aaggcccgcg tgctgggtac cggcgcccgc 720
gcctccgtgc tgtccggcgg caagctggac gcctgggaga agatccgcct gcgccccggc 780
ggcaagaaga agtaccgcct gaagcacctg gtgtgggcct cccgcgagct ggagcgcttc 840
gccctgaacc cctccctgct ggagaccgcc gagggctgcc agcagatcat ggagcagctg 900
cagtccgccc tgaagacctc cgaggagctg aagtccctgt tcaacaccgt ggccaccctg 960
tactgcgtgc accagcgcat cgacgtgaag gacaccaagg aggccctgga caagatcgag 1020
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